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Kin recognition and incest avoidance in a group-living insect

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Mate choice theories predict that animals evolved strategies to mate with optimally genetically dissimilar partners, providing fitness benefits. In group-living species, when adults do not disperse, assessment of relatedness between conspecifics can be a key factor for choosing mates. Here, we report for the first time, kin recognition abilities and their implication in mate choice in the gregarious cockroach, *Blattella germanica* (L.). Binary choice tests showed that females mated preferentially with nonsibling rather than with sibling males, thus avoiding incest. In addition, inbreeding induced an important decrease of their reproductive success. Contrary to what could be expected when females had the choice between a nonsibling strain member and a nonstrain member, they did not avoid mating with distantly related nonstrain members, and extreme outbreeding induced an increase of their reproductive success. Furthermore, our mate choice experiments disentangled the influences of familiarity from those of relatedness and evidenced that kin discrimination was based on genetic cues independently of familiarity. Phenotype matching was a plausible mechanism for kin recognition. Contrary to many insect species, body size was not a salient criterion for mate choice and had no consequences on reproductive success. **Key words:** *Blattella germanica*, group-living insect, incest avoidance, kin recognition, mate choice, reproductive success. [*Behav Ecol* 18:880–887 (2007)]

Mate choice allows individuals of many species to bias their mating toward partners able to provide the best reproductive returns (Andersson 1994). Traditional models of sexual selection predict that in most species, females are more selective than males because of their higher investment in offspring (Bateman 1948; Trivers 1972). Choosing a particular mate could provide females with different kinds of advantages, ranging from direct benefits like courtship feeding or ownership of better foraging territories to indirect benefits of mating with high genetic quality males (Halliday 1983). Body size is often considered to help direct assessment of mate attributes, and, in several species, females prefer larger males because they are more likely to eliminate rivals to control vital resources or to produce sperm in large quantities (Thornhill and Alcock 1983; Andersson 1994). However, animals may also have been selected to mate with an optimally genetically dissimilar partner, bestowing fitness benefits, that is, optimal outbreeding theory (Bateson 1978, 1983; Shields 1983). Both inbreeding and outbreeding can incur fitness costs for parents and/or their offspring. Many authors have demonstrated that crosses between individuals with similar genotypes increase homozygosity of deleterious mutations that can reduce viability, that is, inbreeding depression (Charlesworth D and Charlesworth B 1987; Keller and Waller 2002). Furthermore, crosses between individuals with distant genotypes can also reduce viability by disrupting beneficial gene complexes or local genetic adaptations, that is, outbreeding depression (Price and Waser 1979; Partridge 1983; Edmans 2002). Dispersal of individuals from their natal group over limited distances is a widespread strategy to avoid extreme inbreeding and/or extreme outbreeding (Pusey and Wolf 1996). However, in group-living species with low dispersal rates, the ability to recognize and to discriminate kin among conspecifics can

constitute an alternative strategy when choosing mates (Pusey and Wolf 1996).

To assess genetic relatedness, species possess an internal kin recognition process that can be inferred through kin discrimination, that is, the observable differential treatment of conspecifics, based on cues that correlate with relatedness (Holmes and Sherman 1983). Among the putative mechanisms underlying kin recognition, 4 main theoretical models are generally accepted (Fletcher 1987; Hepper 1991; Mateo 2004), although some authors proposed a different explanatory framework (Barnard 1990; Grafen 1990). The “context-based recognition” model hypothesizes that kin discrimination is based on learning environmental cues so that conspecifics bearing these cues are considered as kin. This is, for example, the case in mother–offspring recognition via natal burrows in ground squirrels (Holmes and Sherman 1982). The “prior association” model hypothesizes that individuals learn the phenotypes of familiar conspecifics during their early development, thus allowing them to discriminate later familiar from nonfamiliar conspecifics. For example, care by helpers in some cooperative bird species is predicted by this prior association better than by genetic relatedness per se (Curry and Grant 1990). The “phenotype-matching” model implies that animals learn their own phenotypes or those of their familiar kin, thus allowing them later to compare, or to match, phenotypes of unknown conspecifics to this learned recognition template (Holmes and Sherman 1982). Individuals can then discriminate kin from familiar conspecifics. For example, in invertebrates, inexperienced field crickets (Simmons 1989) and mites (Enigl and Schausberger 2004) discriminate related from unrelated partners when choosing mates. Finally, kin recognition may be mediated by “recognition-alleles,” that is, green-beard effect (Dawkins 1976). Contrary to the 3 previous models, this mechanism is considered to be independent of learning, and individuals bearing the phenotypic cue encoded by these alleles consider one another as kin. Queen discrimination in fire ants (Keller and Ross 1998) and cooperative aggregation in social amoebas (Queller et al. 2003) constitute 2 of the rare empirical reports supporting this recognition model.

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Many studies show that mate choice based on kin recognition allows avoidance of crossing between closely related partners both in vertebrates and invertebrates. This has been demonstrated, in particular, in some insect species (Maynard Smith 1956; Simmons 1989) including eusocial species (Smith and Ayasse 1987; Keller and Passera 1993; Shellman-Reeve 2001). However, the few results supporting the hypothesis of an optimal balance in outbreeding by avoiding mating with too distantly related partners were obtained only in vertebrates (Bateson 1978; Barnard and Fitzsimons 1988, 1989; Keane 1990). Extreme outbreeding is known to incur fitness costs in some invertebrate species (Burton 1990; Peer and Taborsky 2005; Velando et al. 2006); however, the few studies investigating outbreeding avoidance through behavioral kin discrimination in crickets (Simmons 1991) and copepods (Palmer and Edmands 2000) failed to support this hypothesis. The reasons why so many studies succeeded in demonstrating inbreeding avoidance but failed to evidence outbreeding avoidance could be that, contrary to incestuous mating, opportunities to mate with extremely unrelated partners may be rare in relation to size of home range and movement capacities. Indeed, this mechanism would have no reason to evolve in the absence of frequent interactions among conspecifics from entirely allopatric populations.

Group-living insects with low dispersal rates, but nevertheless with some opportunities to encounter individuals from distant groups, are good models to investigate whether kin recognition influences mate choice so as to regulate the inbreeding/outbreeding balance. We focused on a gregarious cockroach species, *Blattella germanica* (L.), that lives in groups including individuals of all developmental stages and where generations overlap (Ledoux 1945; Roth and Willis 1960). All members of an aggregate share the same shelter and exploit a foraging area around it. In urban habitats, webs of aggregates constitute metapopulations with low migration rates between aggregates and thus between populations (Rivault 1990; Cloarec et al. 1999). Mated females produce oothecae containing fertilized eggs. Approximately 40 diploid full siblings (sex ratio = 1) emerge from each ootheca, and they all reach adulthood and sexual maturity at approximately the same time (Ross and Mullins 1995). The life-history traits of this cockroach thus provide extreme inbreeding opportunities within aggregates and limit extreme outbreeding opportunities between populations. Nevertheless, extreme outbreeding can occur after passive introductions of strangers into local populations through human activity (Cornwell 1968). We hypothesized that kin recognition could constitute a strategy to mate optimally in relation to relatedness in this group-living species, thereby avoiding extreme inbreeding and/or extreme outbreeding. Females usually mate only once in their lifetime (a second mating can occur if the first one is not fertile), storing sperm in a reservoir, that is, the spermatheca, and carrying their successive oothecae until their nymphs (full siblings) hatch, whereas males can remate and do not provide parental care (Cochran 1979). As females invest more than males in reproduction, we hypothesized that they would be more selective.

The aim of this study was to evaluate the influence of kinship on mate choice by females in the gregarious cockroach *B. germanica*. Experiments tested whether sexually receptive females discriminated kin from nonkin partners on the basis of familiarity and/or genetic relatedness. In addition, as in many species females assess male genetic quality by their body size, we tested this parameter as a potential confounding effect in our mate choice experiments. Fitness consequences in relation to mate choice were estimated by reproductive success of females.

MATERIALS AND METHODS

Breeding conditions

Two *B. germanica* laboratory strains, descending from wild individuals, were used (Cloarec et al. 1999). Strain A was collected in Rennes (France, approximately 100 individuals) in 1995 and strain B in Nijni Novgorod (Russia, approximately 20 individuals) in 2000. Both strains were clearly fully crossable and belonged to the same species distributed worldwide (Rust et al. 1995). Each strain was housed in large cages (120 × 80 × 30 cm) where random matings could occur. Strains were kept well isolated one from another. All cockroaches were reared and tested at 25 ± 1 °C under an artificial 12:12 light:dark cycle. They were provided water, turkey food pellets, and cardboard shelters ad libitum. Nymphal development of males was slightly shorter than that of females for both strains under our breeding conditions (males: 55.44 ± 0.34 days; females: 56.85 ± 0.40 days; Wilcoxon, $W = 2990$, $P = 0.0058$).

Experimental individuals

To assess relatedness among experimental individuals, mature oothecae were collected from gravid females and placed individually in glass vials until hatching (Tanaka 1976). Groups of 20 newly hatched first instar nymphs were placed in plastic rearing boxes (80 mm in diameter × 50 mm high) until they became adult. Either 20 nymphs were taken from one ootheca to form one group type or one nymph was taken from each of 20 different oothecae to form another group type. Nymphs from the same ootheca were called “siblings” (knowing they were full siblings), nymphs from 2 different oothecae from the same strain were “strain members,” and nymphs from 2 different strains were “nonstrain members.” As the coefficient of relatedness (r) estimates the probability that 2 individuals share an identical allele by descent (Wright 1922), we assumed that $r = 0.5$ between siblings, $0 \leq r < 0.5$ between strain members, and $r = 0$ between nonstrain members. Individuals bred in the same group during all their nymphal development were “familiar,” and those bred in different groups during this period were “nonfamiliar.” The following 5 categories of adults were obtained from these rearing conditions.

Familiar siblings: individuals from the same ootheca bred together.

Nonfamiliar siblings: individuals from the same ootheca bred in different groups.

Familiar strain members: individuals from different oothecae of the same strain bred together.

Nonfamiliar strain members: individuals from different oothecae of the same strain bred in different groups.

Nonfamiliar nonstrain members: individuals from different strains bred in different groups.

Experimental design

One virgin female was given a simultaneous choice between 2 virgin males, thus forming a test triad. Males were chosen in relation to their familiarity and their relatedness with the female. Each experiment began on the seventh day after the female's imaginal molt, when she became sexually receptive, and lasted until the female died (range: 61–337 days).

Mate choice by females

A color code was used to identify experimental individuals. Each newly emerged adult was marked under CO₂ anesthesia with enamel paint on its pronotum. Adults from a given rearing box were separated by sex before tests to preserve their virginity and maintained in unisexual groups to avoid delaying

sexual maturation, which would occur if they were isolated (Gadot et al. 1989). Six days after their imaginal molt, females were placed individually in an experimental box (80 mm in diameter \times 50 mm high) with water, food, and a piece of cardboard placed upright so that the test cockroaches could use it as resting site and so that the observer could see them all the time. At the same moment, males of the same age were placed individually in small plastic Eppendorf tubes (10 mm in diameter \times 40 mm high) for 24 h. On day 7 after their imaginal molt, just before nightfall, that is, before the beginning of the cockroach activity phase, 2 males were introduced simultaneously into a box containing a female. The males were allowed to walk freely from their Eppendorf tubes into the female's box to form a triad, thus avoiding stress due to CO₂ anesthesia and manipulation by the experimenter. From then on, scan samples were recorded by direct observation at 30 min intervals, night and day, until mating occurred (4.85 ± 0.08 days after the beginning of the test) to identify the successful male. This scan interval was chosen because mating generally lasts more than 1 h (Roth and Willis 1952). During the night phase, scan samples were recorded under red light as it is not perceived by cockroaches (Koehler et al. 1987). Each triad was observed until the female mated (successful test) or produced a sterile ootheca (approximately 6 days after the beginning of the test) because she had not mated (unsuccessful test). After mating, males were removed from the females' boxes to ensure that we analyzed data from a first mating even if it was not fertile (a second mating can occur if the first one is not fertile). Three experiments tested the effects of familiarity and/or relatedness with the male on mate choice by females:

Experiment 1 (familiarity + relatedness effects): females were given a choice between a familiar sibling and a nonfamiliar strain member (212 replicates);

Experiment 2 (familiarity effect): females were given a choice between either a) a familiar sibling and a nonfamiliar sibling (67 replicates) or b) a familiar strain member and a nonfamiliar strain member (108 replicates); and

Experiment 3 (relatedness effect): females were given a choice between either a) a nonfamiliar sibling and a nonfamiliar strain member (93 replicates) or b) a nonfamiliar strain member and a nonfamiliar nonstrain member (106 replicates).

Female fecundity

To estimate female fecundity, 275 successfully mated females (experiment 1: 21; experiment 2a: 41; experiment 2b: 80; experiment 3a: 53; experiment 3b: 80) were maintained isolated in their experimental box until their death (range: 61–337 days). Three types of females were defined in relation to their mate choice: "females in inbred matings" mated with siblings, "females in outbred matings" mated with strain members, and "females in extremely outbred matings" mated with nonstrain members. To estimate their reproductive success, the number of fertile and sterile oothecae each female produced and the number of viable nymphs hatching from each fertile ootheca were recorded daily.

Body size effect

Body size of the members of 130 triads (experiment 1: 21 triads; experiment 2a: 40 triads; experiment 2b: 18 triads; experiment 3a: 51) was estimated. Maximal head width and length of the left mesothoracic femur of each individual were recorded as these measures are correlated with general body size of cockroaches (Lefeuve 1966). Mated and nonmated males were measured at the end of the mate choice experi-

ments, and females were measured after their death. Four types of males (familiar siblings, familiar strain members, nonfamiliar siblings, and nonfamiliar strain members) and 2 types of females (females in inbred matings and females in outbred matings) were measured. Precise measures were obtained by analyzing, using a homemade software (J-P Richard CNRS-UMR 6552), photographs of heads and legs taken under a binocular microscope (25 \times).

Statistical analyses

Data were analyzed using R 2.2.1. (Ihaka and Gentleman 1996). Means are given with standard errors (SEs) (mean \pm SE). Chi-square tests of homogeneity compared proportions of successful tests in different experiments. Binomial tests analyzed binary mate choices (Figure 1). Wilcoxon tests compared body size data (Table 1). One-way ANOVAs and Fisher LSD post hoc tests or analysis of deviance using Chi-square tests and z tests generalized linear model (GLM) procedure; McCullagh and Nelder 1989) assessed the influence of relatedness to mate on life span and fecundity of females (Table 2). Two-way ANOVAs evaluated the influence of size of females, size of males, and interaction between the 2 covariates on the reproductive success of females in inbred matings and in outbred matings estimated by the total numbers of viable nymphs they produced (Table 3).

RESULTS

Mate choice by females

Females from 428 (73.04%) of the 586 test triads mated (successful tests). As proportions of mated females (successful tests) were similar in all experiments (Chi-square test, $\chi^2 = 3.5010$, 4 degrees of freedom [df], $P = 0.4777$), we assumed that mating failures might be due to the physical experimental setup rather than differences in kinship between cockroaches. Under our experimental conditions, the absence of closed harborages, where matings generally occur (Ross and Mullins 1995) may sometimes have interfered with mating. Nonmated females were discarded from further analyses.

Familiarity + relatedness effects

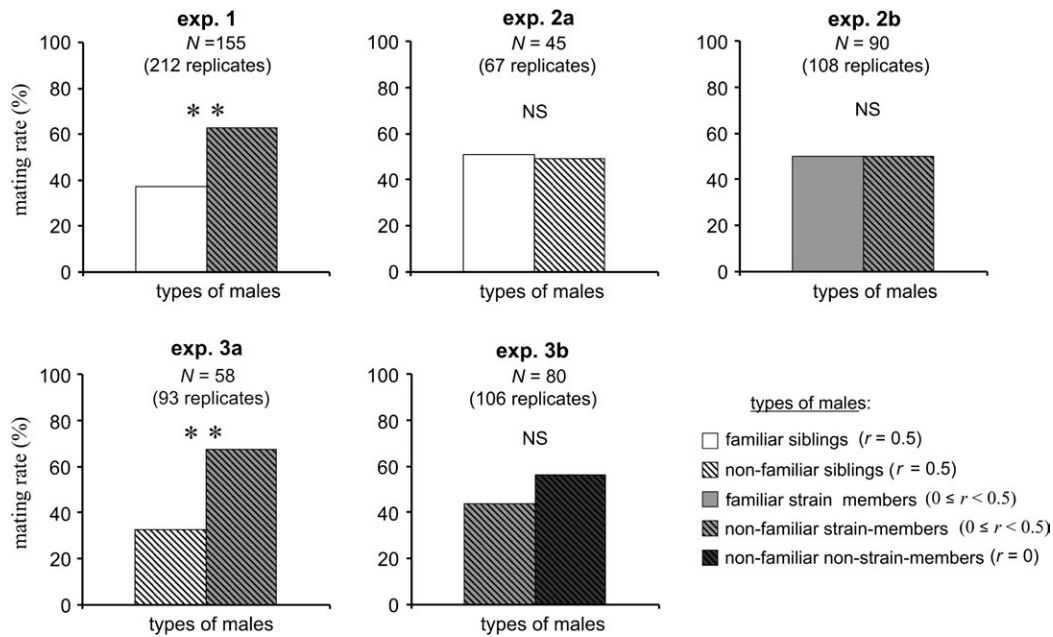
When given a choice between familiar siblings and nonfamiliar strain members (Figure 1, experiment 1), females chose significantly to mate with nonfamiliar strain members (62.58% of the triads; binomial test, $P = 0.0022$). This biased mate choice evidenced that females were able to discriminate between the 2 types of males, either on the basis of familiarity or/and relatedness.

Familiarity effect

Females given a choice between 2 siblings that differed in their familiarity level with the females (Figure 1, experiment 2a) mated as often with familiar as with nonfamiliar males (binomial test, $P = 1.0000$). Similarly, when given a choice between a familiar and a nonfamiliar strain member (Figure 1, experiment 2b), neither type of male was preferred (binomial test, $P = 1.0000$). Therefore, females did not select mates in relation to their familiarity level.

Relatedness effect

Females given a choice between a nonfamiliar sibling and a nonfamiliar strain member (Figure 1, experiment 3a) mated significantly more often with strain members (67.24% of the triads; binomial test, $P = 0.0119$). When given a choice between a nonfamiliar strain member and a nonfamiliar nonstrain member (Figure 1, experiment 3b), females did

**Figure 1**

Proportions of females mating with each type of male in binary choice experiments (exp. 1, 2a, 2b, 3a, and 3b). Males differed in their familiarity level and/or their relatedness with the females. Familiar males: plain color, nonfamiliar males: striped, sibling males: white, strain member males: clear gray, and nonstrain member males: dark gray. *N*: number of successful tests. *r*: estimated genetic relatedness between 2 individuals. Binomial tests: **, $P < 0.005$; NS, $P \geq 0.05$.

not choose significantly either type of male (binomial test, $P = 0.3143$). Nevertheless, data from experiment 3b could be interpreted as a slight, but not significant, preference (56.25% of the triads) for nonstrain members as supported by reproductive success data (see Female fecundity). This result might become significant if the sample was larger. Females clearly avoided inbreeding, but they did not avoid extreme outbreeding.

Male body size effect

To evaluate whether mate choice by females was influenced by male body size, sizes (estimated by head width and femur length) of mated and nonmated males were compared within each of the 4 types of males (Table 1). Male body sizes did not differ significantly between mated and nonmated males in all the 4 types of males (Table 1). Therefore, we concluded that

females did not select their mates in relation to body size of males.

Female fecundity

Isolated mated females survived on average 185.45 ± 2.59 days. Life spans did not differ significantly between females in inbred matings, that is, mated with a sibling, females in outbred matings, that is, mated with a strain member, and females in extremely outbred matings, that is, mated with a nonstrain member (Table 2). Consequently, mate choice did not influence adult life span of females.

Oothecae production

Females produced 5.36 ± 0.08 oothecae on average under our breeding conditions (Table 2). The total number of

Table 1
Body sizes of males and females

Type of individual	Mating success	N	Head width (mm)	P	Femur length (mm)	P
Familiar sibling males	Mated	24	1.99 ± 0.01	0.3757	2.98 ± 0.03	0.2556
	Nonmated	37	1.97 ± 0.01		2.95 ± 0.02	
Familiar strain member males	Mated	9	1.97 ± 0.02	0.8561	2.91 ± 0.03	0.7914
	Nonmated	9	1.96 ± 0.02		2.91 ± 0.03	
Nonfamiliar sibling males	Mated	36	1.95 ± 0.01	0.5231	2.92 ± 0.02	0.1886
	Nonmated	55	1.96 ± 0.01		2.95 ± 0.02	
Nonfamiliar strain member males	Mated	61	1.96 ± 0.01	0.8319	2.94 ± 0.01	0.5637
	Nonmated	29	1.97 ± 0.01		2.92 ± 0.02	
Females in inbred matings	Mated	59	2.09 ± 0.01	0.1619	3.03 ± 0.02	0.1876
Females in outbred matings	Mated	71	2.11 ± 0.01		3.07 ± 0.02	

Head widths and femur lengths (means \pm SE) of mated males, nonmated males, and females from 130 triads (experiment 1: 21 triads; experiment 2a: 40 triads; experiment 2b: 18 triads; experiment 3a: 51 triads) were measured. Data of mated males were compared with those of nonmated males for each of the 4 types of males (defined in relation to their familiarity and/or relatedness with females). Data for females in inbred matings were compared with those for females in outbred matings. *N*, number of individuals measured of each type. *P*, value of Wilcoxon tests.

Table 2
Effect of relatedness between mates on female fecundity

	Females in inbred matings	Females in outbred matings	Females in extremely outbred matings	Statistical analysis	F ratio	P
N	63	167	45	—	—	—
Adult female life span (days)	189.67 ± 5.32	180.96 ± 3.41	196.32 ± 5.19	one-way ANOVA	2.6765	0.0706
Total number of oothecae (fertile + sterile)	5.48 ± 0.16	5.24 ± 0.11	5.58 ± 0.17	one-way ANOVA	1.2702	0.2825
Total number of fertile oothecae	4.30 ± 0.22	4.41 ± 0.11	5.05 ± 0.21	One-way ANOVA	3.7254	0.0254
Total number of sterile oothecae	1.17 ± 0.21	0.86 ± 0.93	0.53 ± 0.15	Deviance analysis (poisson errors)	—	0.0020
Total number of viable nymphs	135.40 ± 7.53	153.40 ± 3.79	170.73 ± 7.67	One-way ANOVA	12.416	0.0005

Duration of adult female life span, total number of oothecae (fertile + sterile), and total number of viable nymphs were recorded and compared for females in inbred, outbred, and extremely outbred matings. N, number of females of each type. Data presented are means ± SE.

oothecae (fertile and sterile) produced during their whole life was not influenced by their mate choice (Table 2). However, relatedness between mating partners influenced the proportion of sterile oothecae produced by females (Table 2). Females in inbred matings produced significantly more sterile oothecae than females in outbred matings (z test, $z = -2.347$, $P = 0.0189$) and females in extremely outbred matings (z test, $z = -3.361$, $P = 0.0008$). Females in outbred matings produced significantly more sterile oothecae than females in extremely outbred matings (z test, $z = -2.047$, $P = 0.0407$). Converse conclusions can be drawn for fertile oothecae (inbred vs. extremely outbred: Fisher LSD, 272 df, $P = 0.0168$; outbred vs. extremely outbred: Fisher LSD, 272 df, $P = 0.0101$), although no significant differences were observed between numbers of fertile oothecae produced by females in inbred matings and by females in outbred matings (inbred vs. outbred: Fisher LSD, 272 df, $P = 0.8234$). The closer females were related to their partner, the fewer fertile oothecae they produced.

The first ootheca of all successfully mated females was fertile (Figure 2). The proportions of females producing a sterile ootheca increased gradually over time and reached 100% for the eighth and ninth oothecae, that is, last ootheca before death. Ootheca production order thus influenced the proportion of females producing sterile oothecae (GLM with binomial errors, $\chi^2 = 6.2790$, $P = 0.0122$). However, the proportions of females producing sterile oothecae did not differ significantly between the 3 types of mating (inbred, outbred, or extremely outbred) for each ootheca order (GLM with binomial errors, $\chi^2 = 3.8191$, $P = 0.1482$). At the ootheca order level, differences related to the 3 types of matings were too small to be significant (Figure 2). We observed a significant effect of relatedness only on the total production of sterile oothecae by each female (Table 2). The observed gradual increase of sterile oothecae over time can be related to sperm depletion in the spermatheca of old females.

Offspring production

Total numbers of viable nymphs produced by females during their entire life were influenced by mate choice (Table 2). Females in inbred matings produced significantly less viable nymphs than females in outbred matings (Fisher LSD, 272 df, $P = 0.0200$) and females in extremely outbred matings (Fisher LSD, 272 df, $P = 0.0006$). Moreover, females in outbred matings produced significantly less offspring than females in extremely outbred matings (Fisher LSD, 272 df, $P = 0.0482$). The closer females were related to their partner, the less offspring they produced.

Two-way ANOVAs analyzed whether the total number of offspring was influenced by body size of mates (estimated by head widths and femur lengths) (Tables 1 and 3). Data con-

cerning females in inbred matings were analyzed separately from those concerning females in outbred matings (Table 3). The total number of viable nymphs per female in inbred matings was not related to her body size, body size of her mate, or to the interaction of the 2 covariates (Table 3). Similar results were found for females in outbred matings (Table 3). Thus, reproductive success was neither influenced by female body size nor by male body size.

DISCUSSION

This study evidenced, for the first time, kin recognition abilities and their implication in mate choice in the gregarious cockroach, *B. germanica*. Our results stressed 1) the impact of relatedness on female mating preference, 2) the implication of kin recognition in incest avoidance, and 3) the consequences of mate choice on individual reproductive success.

Mate choice based on relatedness

Females of many insect species use differences in male body size to bias their mate choice toward larger males that provide higher reproductive returns (Thornhill and Alcock 1983). Our results evidenced that *B. germanica* females did not select males in relation to their body size. Our biometric study revealed a very narrow range of male body sizes (Table 1). Males in this species do not defend territories, and intermale aggressiveness levels are low (Breed et al. 1975). No direct competition with agonistic interactions between the 2 males

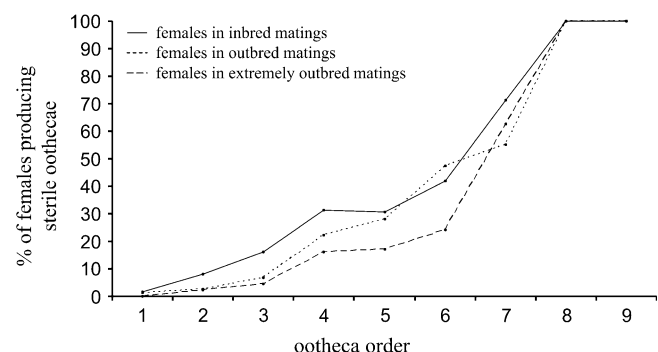


Figure 2
Proportions of females producing sterile oothecae in relation to ootheca order. For each type of female (females in inbred matings: straight line, females in outbred matings: short dashes, and females in extremely outbred matings: long dashes) and each ootheca order, the proportions of females producing sterile oothecae were calculated.

Table 3
Effect of body size of mates (head widths and femur lengths) on female reproductive success

	Total number of viable nymphs			
	Females in inbred matings		Females in outbred matings	
	<i>F</i> ratio	<i>P</i>	<i>F</i> ratio	<i>P</i>
Females head width	0.8030	0.3765	0.3381	0.5641
Males head width	0.0173	0.8960	0.0372	0.8480
Females head width \times males head width	0.0154	0.9020	3.7019	0.0613
Females femur length	0.0856	0.7717	0.1887	0.6663
Males femur length	1.7407	0.1959	2.1393	0.1512
Females femur length \times males femur length	0.1357	0.7149	1.2470	0.2706

Two-way ANOVAs evaluated the influences of female body size, male body size, and the interaction between the 2 covariates on the total number of viable nymphs produced by females in inbred matings and in outbred matings.

of a triad was observed in our experiments. Male body size is clearly not a salient criterion for female mate choice in this species. On the contrary, in the only other cockroach species for which sexual selection and mate choice were studied, *Nau-phoeta cinerea* females mated preferentially with dominant males chosen in relation to their social status (Breed et al. 1980; Moore 1988, 1989). The most important conclusion to be drawn from our biometric data is that body size does not interact with the kinship parameter.

To test the influence of kinship (assessed either by familiarity or by relatedness) on mate choice, *B. germanica* females were given a choice between familiar siblings and nonfamiliar strain members (experiment 1). They showed a preference to mate with nonfamiliar strain members (62.58% of triads), that is, nonsiblings. This kin discrimination revealed that, contrary to male body size, kinship constitutes a major component of mate choice in this species. According to kin recognition models, kinship may be assessed through environmental parameters, familiarity level, and/or genetic relatedness (Fletcher 1987; Hepper 1991; Mateo 2004). As all our experiments were made under identical controlled conditions and the cockroaches were bred on the same diet, they could not discriminate kin on the basis of learned environmental cues, that is, context-based recognition. Furthermore, our data revealed that females did not discriminate familiar from nonfamiliar partners, whether they were their siblings or their strain members (experiment 2a and 2b). Thus, females assessed kinship independently of familiarity, that is, prior association. As they were able to discriminate nonfamiliar siblings from nonfamiliar strain members (experiment 3a), we concluded that cockroaches recognize and discriminate kin on the basis of genetically inherited cues, suggesting either a phenotype-matching mechanism or the use of recognition alleles. Siblings from one ootheca that remain in their natal aggregate constitute a cohort of individuals sexually mature at the same time in the same area (Rivault 1989, 1990). Within aggregates, sexually mature individuals thus encounter familiar conspecifics with various relatedness levels. Therefore, a genetically based recognition mechanism enabling discrimination of siblings among familiar conspecifics is the most adaptive solution to reduce risks of potentially costly discrimination errors linked to inbreeding. As studies focusing on kin recognition mechanisms yielded no clear evidence of genetically encoded templates, learned templates by phenotype matching are suggested in most cases (Waldman 1988; Sherman et al. 1997). Our results revealed that females discriminated siblings from other conspecifics; however, kin recognition through a

green-beard gene would imply an extremely polymorphic locus varying at the ootheca level so that all the siblings from one ootheca, and only them, share the same allele. For all these reasons, we assumed that among putative kin recognition models, phenotype matching is the most parsimonious hypothesis in this group-living species. As for many social insects, cuticular odors could be good candidates for kin recognition cues in *B. germanica* (Singer 1998; Vander Meer and Morel 1998; Lenoir et al. 1999).

Incest avoidance through kin recognition

Females given a choice between a sibling and a strain member biased their mate choice toward strain members (experiment 1 and 3a), thus avoiding inbreeding, that is, crosses between siblings. Furthermore, when given a choice between strain members and nonstrain members, they did not avoid extreme outbreeding. Our data did not allow us to conclude that females favored extreme outbreeding, that is, crosses between nonstrain members (experiment 3b), although a slight (but not significant) preference for nonstrain members was observed. We cannot exclude that a significant preference could be observed with a larger sample size. The fact that females did not avoid pairing with distantly related partners could be explained by a lack of fitness costs for mating with nonstrain members. *Blattella germanica* populations may not be submitted to environmental pressures strong enough to favor major local adaptations. The few studies investigating the genetic structure of field populations revealed no significant differences between geographically distant strains (Cloarec et al. 1999). Although *B. germanica* are distributed worldwide in human habitats, all their indoor microhabitats are extremely similar (Appel 1995). Favorable coadapted gene complexes and local adaptations that risk disruption by extreme outcrossing are probably rare in wild populations of this species. Thus, the strain members and nonstrain members tested in experiment 3b are probably not sufficiently genetically different to induce extreme outbreeding costs although our strains originated from locations approximately 4000 km apart.

Blattella germanica can be added to the few insect species for which incest avoidance through kin discrimination during mate choice has been reported (Maynard Smith 1956; Smith and Ayasse 1987; Simmons 1989; Keller and Passera 1993; Shellman-Reeve 2001). In addition to kin recognition, some species have developed different strategies to avoid incestuous mating in relation to their ecology and to their social organization like dispersal, extrapair copulation, or even delayed

maturation between sexes (Blouin SF and Blouin M 1988; Pusey and Wolf 1996). None of these mechanisms apply to *B. germanica*. Adults of both sexes are philopatric (Rivault 1989, 1990) and a single mating provides sufficient sperm to last during the entire reproductive life of a female (Cochran 1979) so that multiple matings and extrapair copulations are rare. Although nymphal development of males lasted one day less than that of females under our experimental conditions, sexual maturation is synchronous within a cohort. For all these reasons, kin discrimination constitutes the main inbreeding avoidance mechanism in this gregarious species, favoring gene flow within an aggregate. As females did not avoid mating with nonstrain members (experiment 3b), gene flow may also occur between aggregates or between populations through extreme outbreeding. The fact that individuals from distant populations are regularly introduced into local populations through human activity would facilitate outcrossing, increase genetic diversity, and ensure population stability.

Consequences on reproductive success

Our results evidence that female fecundity is related neither to their own body size nor to the body size of their mate. Contrary to many insect species where larger females possess higher quantities of nutritive reserves or larger males provide larger sperm quantities (Thornhill and Alcock 1983; Simmons 1988; Fox et al. 1995; Savalli and Fox 1998; Jiménez-Pérez and Wang 2004), body size did not influence individual reproductive success in *B. germanica*. This result is not surprising because size differences between individuals of the same sex were very small.

However, our study revealed differences in fecundity of females that were due to relatedness between mates. Although the total numbers of oothecae produced by mated females did not differ between experimental conditions, females in inbred matings produced more sterile oothecae than females in outbred and in extremely outbred matings. Inbred pairs produced approximately 12% less offspring than outbred pairs. This decline of direct fitness is congruent with inbreeding depression observed in the first generation in many insect species (Maynard Smith 1956; Henter 2003; Fox and Scheibly 2006). Contrary to the decrease expected after extreme outbreeding (Price and Waser 1979; Partridge 1983; Edmans 2002), we observed that extremely outbred pairs produced 11% more offspring than outbred pairs. Crossings between nonstrain members were thus more fertile than crossings between strain members. These data suggest that our laboratory strains were highly inbred, probably because they were founded by a small number of individuals. This observed increase in fitness, in addition to the fact that females did not avoid mating with nonstrain members (experiment 3b), led us to conclude that extreme outbreeding would be adaptive and should be favored in this species.

Whatever their mate choice, the first ootheca produced by all the successfully inseminated females was fertile. Later, toward the end of their lives, they produced only sterile oothecae. This result indicates gradual depletion of sperm in female spermathecae. The observed differences in female fecundity suggest a different investment in the size of ejaculate of males in relation to their relatedness with females rather than genetic inbreeding and/or outbreeding effects. Males probably adapt their mating effort in relation to their relatedness with their partner. Because no behavioral studies describe the precopulatory sequences between males and females, the reported experiments did not allow us to disentangle female mate choice from male mate choice, and we cannot exclude male selectivity. Costs of mating for males, like sperm production or courtship effort, may have been underesti-

mated in this species. When mating costs are high for males, mate choice theories predict that they become selective and are able to bias their mating effort toward females with the highest reproductive values (Bonduriansky 2001).

To conclude, this study highlights the importance of mate choice on reproductive success in *B. germanica*. As far as we know, this is the first study showing a mating distribution skewed toward nonsiblings in a cockroach species, indicating that kin recognition and relatedness are key factors in mating strategies. Kins are discriminated on the basis of relatedness, independently of familiarity, and so far phenotype matching is a plausible mechanism of kin recognition. Kin discrimination constitutes the main incest avoidance mechanism in this species and could favor gene flow within and among wild populations. Studying recognition systems and mating strategies in group-living insects presents a primordial interest to understand their social organization and population dynamics.

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